

First lichenicolous records of *Chaetopyrena penicillata*

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ABSTRACT—*Chaetopyrena penicillata* is reported for the first time as a lichenicolous fungus. A culture and a sequence were obtained from material growing on *Xanthoria parietina*. Features of the culture on PDA and MEA, the ecology and geography, and the phylogenetic position within *Didymellaceae* based on an ITS sequence are given. A key to the lichenicolous coelomycetes with setose pycnidia is also provided.

KEY WORDS—coelomycete, *Pleosporales*, setose fungi, Southern Ukraine

Introduction

The lichenized fungus *Xanthoria parietina* (L.) Th. Fr. is a fine model for the study of the diversity and ecology of lichenicolous fungi (Etayo & Berger 2009, Fleischhacker 2011, Braun & al. 2016, Khodosovtsev & Darmostuk 2016, Tsurukau & Etayo 2017). In 2016, we collected *Pyrenochaeta*-like specimens on apothecia of corticolous *Xanthoria parietina* for testing their growth in culture. Surprisingly, we found a fungus with ampulliform conidiogenous cells and large bacilliform conidia that represented neither *Pyrenochaeta xanthoriae* Diederich (Diederich 1990) nor *Pyrenochaetopsis* Gruyter & al. (de Gruyter & Boerema 2002). Instead, morphological, cultural, and molecular data allowed

us to identify the fungus as *Chaetopyrena penicillata*. Further research has revealed the presence of this species on *Peltigera rufescens* (Weiss) Humb. and *Physcia stellaris* (L.) Nyl.

Chaetopyrena Pass. is a poorly studied asexual genus, and its phylogenetic position in *Didymellaceae* has only recently been identified (de Gruyter & al. 2010). No modern description of the genus was provided in Hyde & al. (2013), nor are any molecular data available from the generic type, *Chaetopyrena hesperidum* Pass., described from *Citrus* (Hyde & al. 2013). Only one *Chaetopyrena* species, *C. penicillata*, has been isolated in culture for which ITS and LSU sequences have also been obtained (Arzanlou & Khodaei 2012; Wang & al. 2016). In this paper, we report for the first time a species of *Chaetopyrena* with a lichenicolous habit and provide a full description, cultural characteristics, and ITS sequence data.

Material & methods

Morphological observations and isolation

The material was examined using standard microscopic techniques. Sections for anatomical examination were cut by hand and studied microscopically in water preparations. Measurements were made in water with an accuracy of 0.2 µm for conidia, conidiogenous cells, cell walls and hyphae; of 5 µm for the pycnidial wall; and of 10 µm for conidiomata. Measurements are given as (min.–) x –SD – x +SD (–max.), where x is the average and SD the standard deviation. Photographs were taken with a Levenhuk C510 NG camera on an Optica Italica stereomicroscope and MICROMED-2 microscope. All examined specimens are deposited in the lichenological herbarium of the Kherson State University, Ivano-Frankivsk, Ukraine (KHER) and in the personal herbarium of the first author (herb. VD).

Pure cultures were obtained from a multiconidial culture (Bomar & Knöpfel 1992). Malt extract agar (MEA) and potato dextrose agar (PDA) were used for isolation of the fungal colonies (Crous & al. 2009). Fungi isolates were deposited in culture collection of Kherson State University, but they are currently unavailable for research.

DNA extraction, amplification and sequencing

Fungal genomic DNA was extracted from fresh mycelium and pycnidia grown on PDA at 25°C for 2 months using a modified CTAB-method (Doyle & Doyle 1990, Tarieiev & al. 2011). The internal transcribed spacer (ITS) region was PCR amplified and sequenced using universal primers ITS1–ITS4 and ITS4–ITS5 according to White & al. (1990). The PCR cycle protocols followed Ekman (2001). PCR products were visualized on a 1% agarose gel using ethidium bromide. Purification and sequencing of the PCR amplicons with ITS1 and ITS4 primers was conducted at Macrogen Inc. (<http://www.Macrogen.com>, The Netherlands).

TABLE 1. Strains and sequences used in the phylogenetic analyses.
The new sequence is indicated in **bold**. Type vouchers are annotated as [T].

TAXON	VOUCHER	HOST	ITS
<i>Ascochyta pisi</i>	CBS 108.26	—	MH854853
	Netherlands, CBS 122785 [T]	—	GU237763
	Iran, MoKhol3-2	<i>Lathyrus sativus</i>	MT351037
<i>Calophoma clematidina</i>	Netherlands, CBS 108.79	—	MH861182
	Netherlands, CBS 520.66	—	MH858873
	Netherlands, CBS 108.79 [T]	—	FJ426989
	Netherlands, CBS 201.49	—	FJ426991
	Netherlands, CBS 195.64	<i>Clematis</i> × <i>jackmanii</i>	FJ426990
	Netherlands, CBS 520.66	<i>Selaginella</i> sp.	FJ426992
	Ukraine, KHER 10840	<i>Xanthoria parietina</i>	MW478633
<i>Chaetopyrena penicillata</i>	Iran, Khodaei P4I1	<i>Prunus divaricata</i>	MK100126
	Iran, Arzanlou S5	<i>Elaeagnus angustifolia</i>	MK100127
	Iran, Khodaei T312I1	<i>Salix alba</i>	MK100128
	Iran, Khodaei T22I1	Plant litter	MK100129
	China, HGAU-091001	—	KC492443
	CCTU 260	<i>Elaeagnus angustifolia</i>	JQ663990
	France, CBS 183.55 [T]	<i>Rumex arifolius</i>	EF192139
<i>Didymella exigua</i>	France, CBS 183.55 [T]	<i>Rumex arifolius</i>	EF192139
<i>D. pisi</i> (= <i>Ascochyta pisi</i>)	—	—	GU722316
<i>Neoascochyta exitialis</i>	Switzerland, CBS 389.86	—	MH861971
	Sweden, CBS 113693	<i>Allium</i> sp.	KT389513
	CBS 118.40	—	KT389514
	Netherlands, CBS 389.86	<i>Triticum aestivum</i>	KT389515
	Germany, CBS 811.84	<i>Secale cereale</i>	KT389516
	Germany, CBS 812.84	<i>Hordeum vulgare</i>	KT389517
<i>Phoma clematidina</i> (≡ <i>Calophoma clematidina</i>)	Netherlands, PD 95.895	<i>Clematis</i> sp.	FJ515599
<i>P. herbarum</i>	C61	—	JQ936277
	C108.1	—	JQ936274
	C28.4	—	JQ936275
<i>Pyrenochaeta nobilis</i>	Italy, CBS 407.76 [T]	—	EU930011
	CBS 292.74	—	MH860856
<i>Pyrenochaetopsis leptospora</i>	CBS 101635 [T]	—	JF740262
<i>P. microspora</i>	Montenegro, CBS 102876	—	MH862809
	Brazil, PB147	<i>Cocos nucifera</i>	MK508814

Sequence alignment and phylogenetic analysis

The quality of the newly produced sequence was manually checked using sequence chromatogram in Chromas software (Technelysium Pty Ltd; <http://www.technelysium.com.au/chromas.html>) and edited in BioEdit 7.2.5 (Hall 1999). We used a BLASTN search (Altschul & al. 1990) in the GenBank database for primary taxonomic interpretation of the sequence. The final analyses included the newly generated sequence and available NCBI accession number sequences with complete ITS1 region of *Chaetopyrena* and selected genera of *Didymellaceae* such as *Ascochyta*, *Calophoma*, *Didymella*, *Neoascochyta*, *Phoma* (TABLE 1). *Pyrenochaetopsis leptospora* (Sacc. & Briard) Gruyter & al. was used as outgroup. The ITS region was aligned using MAFFT 7 (Katoh & Standley 2013) with L-INS-i method (Katoh & al. 2005). The final ITS alignment contained 456 positions and 36 sequences. To determine the evolutionary models that fit best for the data set, the program jModeltest 2.1.7 (Darriba & al. 2012) was used. The best nucleotide substitution model GTR+G+I (Tavaré 1986) was selected using the Maximum Likelihood value ($-\ln L$) criterion (Posada & Crandall 1998). Phylogenetic reconstruction of the resulting alignment was carried out using the Metropolis-coupled Markov chain Monte Carlo (MCMC) approach in MrBayes v.3.2 (Ronquist & al. 2012). Two parallel simultaneous runs, each using four independent chains and starting from a random tree, were performed over 10 000 000 generations; tree sampling was carried out every 1000th generation. The first 25% of saved data was discarded as burn-in and the 50% majority-rule consensus tree and posterior probabilities (PP) were calculated from the remainder. A maximum likelihood (ML) approach was applied to the same data using IQTree Web Server (Trifinopoulos & al. 2016) with the GTR evolutionary model selected. Non-parametric bootstrap analysis was performed with 1000 ultrafast bootstrap replicates. The maximum likelihood consensus tree is not shown, but bootstrap values (BS) are indicated at branches in the Bayesian tree. Well supported clades were considered with PP >0.95 and BS >70. The alignment and tree used in this study are publicly available in TreeBase (ID: 27741). The final tree was visualized and modified in FigTree v.1.4.4 and Inkscape v.1.0.2 software (<https://inkscape.org/>, Rambaut & Drummond 2018).

Phylogenetic results

PHYLOGENY. Maximum likelihood (ML) and Bayesian trees had no topological conflicts in the clades. Newly generated and other sequences of *Chaetopyrena penicillata* formed a well-supported monophyletic clade (PP = 1, BS = 100) within *Didymellaceae* and were sister to *Calophoma* Qian Chen & L. Cai (type species *Calophoma clematidina* (Thüm.) Qian Chen & L. Cai) and *Ascochyta* Lib. (type species *Ascochyta pisi* Lib.) (FIG. 1).

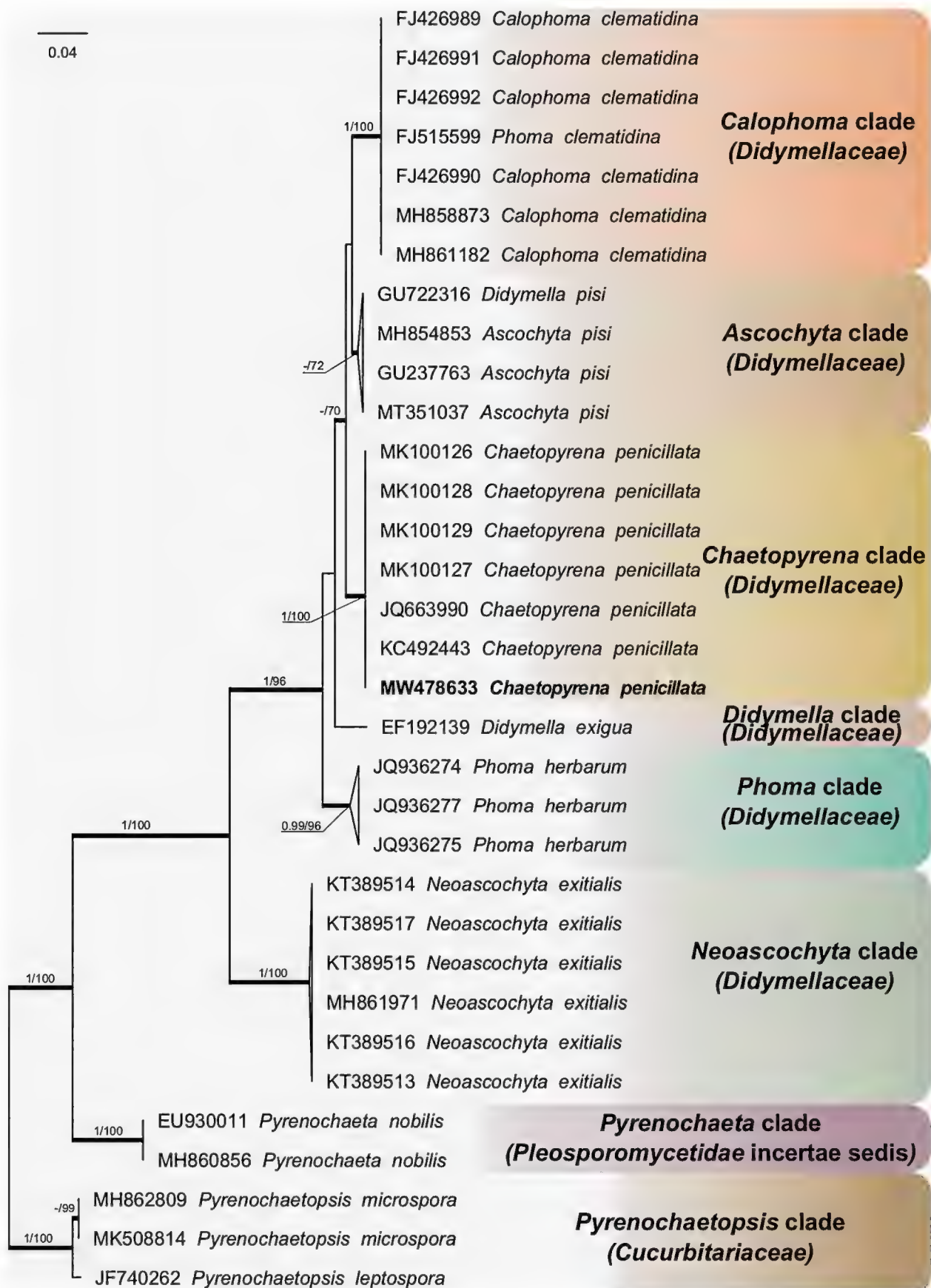


FIG. 1. Internal transcribed spacer (ITS)-based 50% majority-rule unrooted consensus tree based on a Bayesian approach for *Chaetopyrena penicillata*. Bold branches represent either bootstrap values ≥ 70 and/or Bayesian posterior probabilities ≥ 0.97 . Newly generated sequence is indicated in bold.

Taxonomy

Chaetopyrena penicillata (Fuckel) Höhn., Hedwigia 60: 132 (1918) FIG. 2

DESCRIPTION IN VIVO (from specimen on *Xanthoria parietina*)—Conidiomata pycnidia, globose to ellipsoid, fully immersed at first, emerged up to superficial when mature, dark brown to brown-black, (90–)105–140 (–150) × (80–)85–115 (–125) µm [*n* = 20]; pycnidia covered around the ostiole with erect, brown, 4–5-septate setae, (65–)80–130 (–150) × (4.0–)5.0–6.6 (–7.2) µm [*n* = 20]; pycnidial wall of 3–4 layers (textura angularis), (15–)18–32 (–35) µm [*n* = 20] thick, cells (8.4–)9.6–13.8 (–15.4) × (6.0–)7.4–9.8 (10.2) µm [*n* = 25], with an amorphous brown pigment in cellular walls; conidiophores reduced to conidiogenous cells or with a single supporting cell, ampulliform, hyaline, smooth, with periclinal thickening, (5.2–)6.4–8.2 (–9.0) × (4.2–)5.4–7.6 (–8.4) µm [*n* = 20]; conidia solitary, 1-celled, hyaline, smooth, cylindrical with rounded ends, often slightly constricted in the middle (dumbbell-shaped), (11.6–)13.0–15.6 (–16.4) × (2.2–)3.2–3.8 (–4.4) µm [*n* = 60].

DESCRIPTION IN VITRO (PDA) – Vegetative hyphae (3.2–)4.2–6.6 (–7.2) µm wide [*n* = 25] with oil drops, conidiomata pycnidia, subglobose to pyriform, initially hyaline (3–5 days), then dark brown to brown-black (14 days), erumpent with one or few (2–3) necks, (150–)230–300 (–360) µm wide and (300–)340–440 (–520) µm high [*n* = 30]; neck (90–)120–240 (–300) µm long [*n* = 20] µm long, with a central ostiole, ≤30 µm diam., setose; setae 2–3-septate, medium brown, numerous, erect, smooth, tapering towards hyaline to pale brown obtuse ends, (75–)100–170 (–225) × (3.0–)5.2–8.4 (–9.6) µm [*n* = 40]; pycnidial wall of 3–5 layers (textura angularis), (30–)40–50 (–60) µm [*n* = 30] thick, internal layer thin, hyaline to pale brown, outer layer wider, medium brown to dark brown; cells (5.2–)8.2–14.4 (–18.4) × (4.0–)6.2–10.8 (–14.2) µm [*n* = 40], with an amorphous brown pigment in cellular walls; conidiophores reduced to conidiogenous cells or with a single supporting cell, ampulliform, hyaline, smooth, with periclinal thickening, (6.2–)7.2–9.8 (–10.4) × (5.2–)7.6–9.8 (–10.0) µm [*n* = 20]. Conidia solitary, 1-celled, hyaline, smooth, cylindrical with obtuse ends, rarely pyriform to slightly constricted in the middle, (12.0–)13.0–14.6 (–15.4) × (2.6–)3.2–4.6 (–5.6) µm [*n* = 60].

CULTURE CHARACTERISTICS – On MEA, colonies flat, ≤2.5 cm diam. after 7 days, spreading with sparse hyaline aerial mycelium, hyaline to pale brown internal mycelium, even, smooth margins, surface dirty white to pale brown,

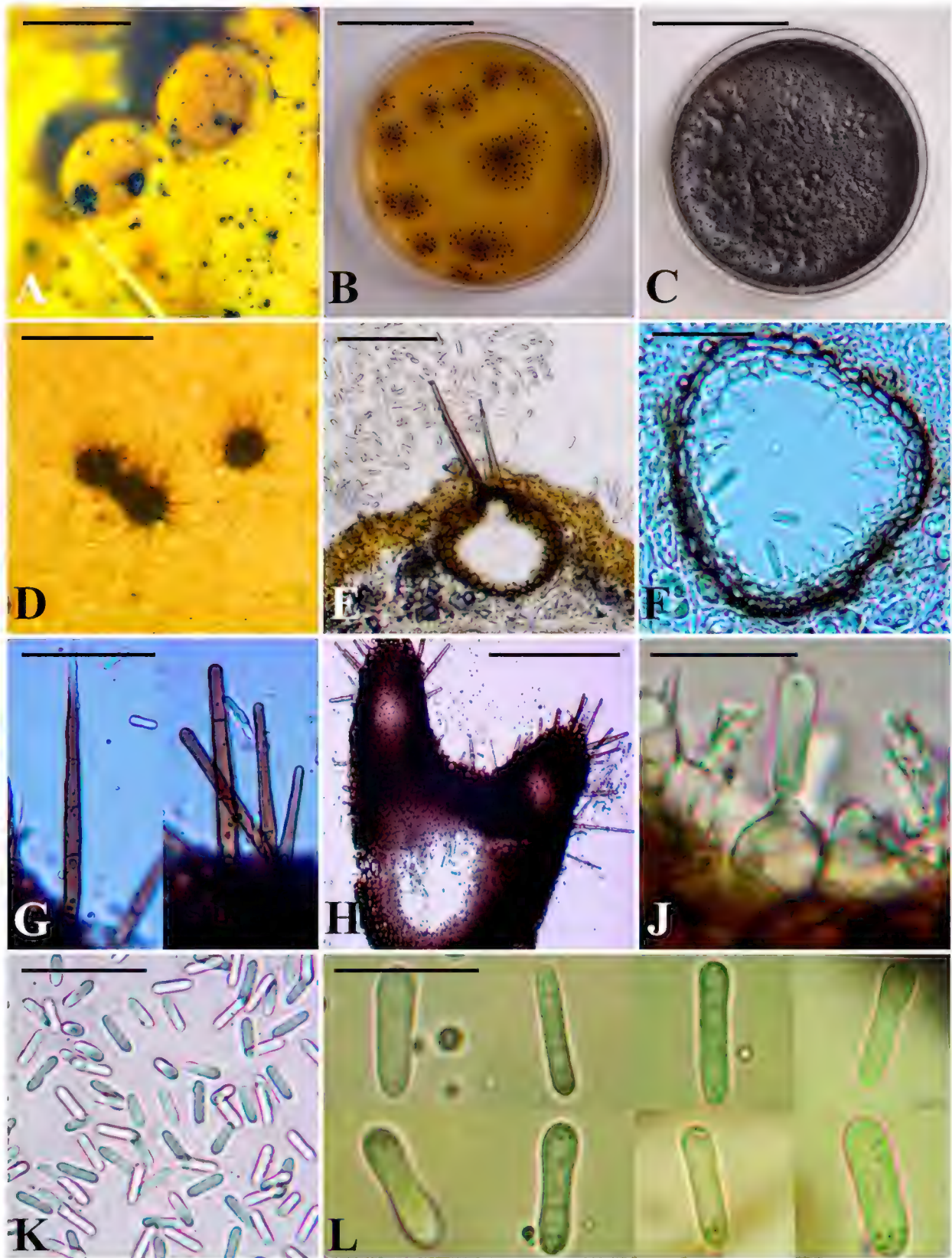


FIG. 2. *Chaetopyrena penicillata* (KHER 10840): A. pycnidia on apothecia of *Xanthoria parietina*; B. one month old culture (MEA); C. one month old culture (PDA); D. pycnidia (MEA); E. section through pycnidium (in water); F. pycnidial wall, conidiogenous cells, and conidia (in water); G. setae (in water); H. apical taper of pycnidium (in water); J. ampulliform conidiogenous cells and conidium (in water); K, L. conidia (in water). Scale bars: A, D = 1 mm; B, C = 5 cm; E, H = 100 μ m; F, G, K = 50 μ m; J = 25 μ m; L = 15 μ m.

similar in reverse. On PDA, colonies flat, ≤ 6 cm diam. after 7 days, spreading with hyaline to pale brown sparse aerial mycelium, medium brown to dark brown internal mycelium, even, smooth margins, surface olivaceous-grey to lead-black, olivaceous-black in reverse.

SPECIMENS EXAMINED – UKRAINE. KHERSON REGION. Skadovskyi district, village Kardashynka, private summer house, 46.5548°N 32.6463°E, alt. 1 m, on *Xanthoria parietina*, on *Salix alba*, 28.XII.2015, A. Khodosovtsev (KHER 10840; culture KHER_56 NCBI accession number MW478633); MYKOLAIV REGION, Pervomaiskyi district, near Kuripchyne village, National Nature Park Buzkyi Gard, 47.9953°N 31.0017°E, alt. 48 m, on *Peltigera rufescens*, on soil, 21.IX.2019, V. Darmostuk (herb. VD 281); Voznesenskyi district, near Vysoka Hora village, Dubova balka, 47.8908°N 31.6131°E, alt. 37 m, on *Physcia stellaris* and *X. parietina*, on *Fraxinus* twig, 21.X.2020, V. Darmostuk (herb. VD 906).

ECOLOGY—*Chaetopyrena penicillata* was originally described from stems of *Medicago sativa* (Fuckel 1867) and subsequently reported as a saprotroph on dry leaves of *Medicago* spp. and on fruits of *Elaeagnus angustifolia* showing dry rot symptoms (Arzanlou & Khodaei 2012), endophytic in stems of *Ephedra intermedia* (Wang & al. 2016), and from leaves of *Fraxinus chinensis* (Vettraino & al. 2017). Moreover, it is known as a saprophyte on soil, dead twigs, fruit, and stubble (Wang & al. 2016). Here it is reported for the first time growing on the lichens *Peltigera rufescens*, *Physcia stellaris*, and *Xanthoria parietina*, forming pycnidia on weakened thalli and apothecia in wet seasons. The infection of lichens by *Chaetopyrena penicillata* does not induce gall formation, but sometimes (depending on the host species) causes a discoloration of the host thallus; on *Xanthoria parietina* it does not induce discoloration, but we observed totally discolored thalli on *Physcia stellaris*, and a few necrotic parts on *Peltigera rufescens*.

DISTRIBUTION—Specimens on *Physcia stellaris* and *Peltigera rufescens* have the same morphological features. Our data fit the measurements given by other authors (Fuckel 1867, Arzanlou & Khodaei 2012, Wang & al. 2016). The fungus is known from Europe (Czech Republic, Germany, Romania, Russia), Asia (China, Iran, Turkey), and Africa (South Africa) (Fuckel 1867, Arzanlou & Khodaei 2012, Wang & al. 2016). It is here reported from Ukraine for the first time.

COMMENTS—Visually, *Chaetopyrena penicillata* is similar to *Pyrenochaeta xanthoriae*, which can be distinguished by acro-pleurogenous conidiogenous cells and shorter conidia (Diederich 1990). Another lichenicolous coelomycete with setose pycnidia “*Pyrenochaeta*” *collematis* Vouaux has cylindrical

conidiogenous cells and smaller conidia and grows on thalli of *Enchylium tenax* (Hawksworth 1981, Clauzade & al. 1989). This species is dubious due to the lost type and lack of modern records and description. The facultatively lichenicolous *Pyrenochaetopsis microspora* (Gruyter & Boerema) Gruyter & al. (*Cucurbitariaceae*) differs by much smaller conidia ($3.5\text{--}4.5 \times 1.5\text{--}2 \mu\text{m}$ vs. $12\text{--}15.5 \times 2.6\text{--}5.7 \mu\text{m}$ in *C. penicillata*; de Gruyter & Boerema 2002). The asexual stage of *Coniothyrium sidae* Quaedvl. & al. (*Coniothyriaceae*) differs in slightly smaller ($9\text{--}13 \times 2.5\text{--}3 \mu\text{m}$) conidia and smaller ($4\text{--}7 \times 4\text{--}6 \mu\text{m}$) conidiogenous cells and grows on the angiosperm *Sida* (Quaedvlieg & al. 2013). The setose coelomycetous *Paraphoma* Morgan-Jones & J.F. White and *Setophoma* Gruyter & al. belong to different clades within *Phaeosphaeriaceae* (de Gruyter & al. 2010) and have slight morphological differences. *Dinemasporium strigosum* (Pers.) Sacc., a common coelomycete, growing mostly on dead grasses but also reported from *Peltigera* thalli, differs in producing conidia with a single filiform appendage at each end (Sutton 1980, Nag Raj 1993, Sérusiaux & al. 2003). Lichenicolous coelomycetes with setose pycnidia are also known in *Karsteniomyces* D. Hawksw. and *Keratosphaera* H.B.P. Upadhyay (Upadhyay 1964, Boqueras & Diederich 1993, Matzer 1996).

Key to the lichenicolous coelomycetes with setose pycnidia

1. Setae light brown to brown 2
1. Setae hyaline, conidiophores cylindrical, 30-40 μm long, conidiogenous cells monoblastic, indistinguishable from conidiophores, conidia hyaline, elongate, 1-septate, on *Parmelina quercina* *Karsteniomyces llimonae*
2. Conidia with filiform apical appendages 3
2. Conidia without filiform apical appendages 7
3. Conidia $\leq 20 \mu\text{m}$ long 4
3. Conidia 25-30 μm long, with a single filiform appendage at each end, on *Peltigera* and (rarely) other (foliose) lichens *Dinemasporium strigosum*
4. Conidia 0-1-septate 5
4. Conidia 2-4-septate, on *Mazosia phyllosema* *Keratosphaera batistae*
5. Setae unforked at the distal end 6
5. Setae one or twice shortly forked at the distal end, on *Porina epiphylla* *Keratosphaera furcatiseta*
6. Apical part of setae distinctly pointed and paler, $35 \times 8 \mu\text{m}$, on *Porina epiphylla* and *Pyrenula nitida* *Keratosphaera porinae*
6. Apical part of setae not pointed and not paler, $16 \times 4 \mu\text{m}$, on *Dimerella* spp. *Keratosphaera dimerellae*

7. Conidiogenous cells cylindrical, 1.5–2.5 μm diam. 8
7. Conidiogenous cells ampulliform, 5–10 μm diam. 9
8. Conidiogenous cells arising from elongate septate conidiophores,
conidia 3–3.5(–4) $\times$ 1.4– 1.8(–2) μm , on *Xanthoria* *Pyrenochaeta xanthoriae*
8. Conidiophores reduced to the conidiogenous cell,
conidia 5–6 $\times$ 2 μm , on *Enchylium* “*Pyrenochaeta*” *collematis*
9. Conidia 3.5–4.5 $\times$ 1.5–2 μm ,
on *Buellia* *Pyrenochaetopsis microspora*
9. Conidia 12–15.5 $\times$ 2.6–5.7 μm ,
on *Peltigera*, *Physcia*, and *Xanthoria* *Chaetopyrena penicillata*

Acknowledgements

We wish to thank Wolfgang von Brackel (Vegetationskundlich-ökologische Gutachten & Lichenologie, Röttenbach, Germany), Paul Diederich (Musée national d’histoire naturelle, Luxembourg), and Ave Suija (Institute of Ecology and Earth Sciences, University of Tartu, Estonia) for constructive comments on the manuscript and Anna Naumovich and Viktoriya Ovsienko (National Nature Park “Nyzhniodniprovskiy”) for the help with specimen isolation and DNA extraction. This study was financially supported by the project of Ministry of Science and Education of Ukraine N 0116U004735.

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